Effects of Pollution on the Free Amino Acid Content of Two Marine Invertebrates

RITA D. SCHAFER[1]

RECENT REPORTS have been received from some inhabitants of the southern California coastal area of a change in the texture and taste of certain edible marine invertebrates. Investigation showed that the specimens so designated had been taken from areas known to be polluted. The term "polluted" as used here has been given the same meaning as that used in the ecological study by Reish (1956) on the San Gabriel River area: this meaning is the dictionary definition, "the act of making or rendering unclean."

It has been shown for some invertebrates that a change in environmental factors will produce a change in either histological or morphological composition. Kinne (1958) showed that a change in ectodermal cell shape can be induced in *Cordylophora craspia* by varying the environment from marine to brackish. Wilson and Armstrong (1958), after experimentation, concluded that *Echinus* eggs and larvae are affected structurally by the properties of sea water. It has also been demonstrated (Lane and Schafer, in progress) that a difference in diet may change the amino acid composition of muscle tissue in some invertebrates. Since this is known to be true under controlled conditions, the possibility exists that a variation in tissue composition might occur as a result of a polluted and consequently altered environment.

This study was thus undertaken to determine (1) if the change in appearance and taste was accompanied by a change in amino acid composition, and (2) the nature of the change, if one had occurred.

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MATERIALS AND METHODS

The animals used for investigation were the abalone, *Haliotis cracherodii* Leach, and the crab, *Pachygrapsus crassipes* Randall. The abalone was selected for study, as it is the form in which the greatest difference in tissue texture and taste has been observed. *P. crassipes* was selected because of its wide distribution and marked tolerance of polluted conditions. These two forms also offer two extremes as to length of time in which the animals are directly subjected to the polluted water. *Haliotis* is exposed to the air only during periods of lower low tides and therefore is almost continuously within the influence of the conditions prevailing in the polluted water. *Pachygrapsus,* on the contrary, lives in the high tide zone along a rocky shore or on floats rather than in the water in a wharf region and consequently is submerged only during periods of high tide, and is directly subjected to the pollutants for comparatively less time than is the abalone. Collections were made from polluted waters and, for a basis of comparison, from waters known to be free of pollution.

Specimens of *Haliotis* were collected along the shore at White's Point in the Palos Verde region of the southern California coast, from San Clemente Island, Anacapa Island, and Santa

FIG. 1. Areas of collection.

Catalina Island. *Pachygrapsus crassipes* were collected from a point in the west channel of the Los Angeles Harbor area designated as L.A. 7, the mouth of the San Gabriel River at Alamitos Bay, White's Point, Santa Catalina Island, Anacapa Island, and Morro Bay (Fig. 1). White's Point, L.A. 7, and the mouth of the San Gabriel River at Alamitos Bay can be considered polluted areas; while Morro Bay, Santa Catalina Island, San Clemente Island, and Anacapa Island are surrounded by nonpolluted water. In the polluted areas both the nature and the degree of pollution vary.

White's Point is a sewer outfall area in which the pipes convey the treated sewage to a site 6,716 ft. from the shore line. According to Stephenson and Grady (1956), oxygen deficiency at 6,000–8,000 ft. from the outfall varied from 10–30 per cent, at one time of measurement, to 0 per cent (or normal) at another time. It was reported, further, that ammonia content was not normal in any area within 11,000 ft. of the outfall. Measurements taken at 3,000 ft. from the outfall showed an increase of silicates and phosphates. Although dilution undoubtedly occurs in the remaining 3,700 ft., it would not be safe to assume that the water in the intertidal area where the specimens were taken is of normal composition.

The White's Point area is divided at low tide into a northern cove and a southern cove by a narrow expanse of rock which extends out from shore for approximately 200 ft. A survey of the fauna of these two sections shows them to be quite different. On the southern side of the rocky projections were found scattered specimens of *Haliotis cracherodii,* an occasional *Pachygrapsus crassipes,* masses of the tubed worm, *Phragmatopoma californica,* and specimens of the limpets *Fissurella volcano* and *Acmaea limatula.* Empty *Olivella* shells were also present in relatively large numbers. In sharp contrast to this paucity of forms and individuals was the fauna of the northern section. Here were noted specimens of *Pisaster ochraceus, Strongylocentrotus purpuratus, S. franciscanus, Ophiothrix spiculata, Bulla gouldiana, Aplysia californica, Octopus bimaculatus, Conus californicus, Pagurus samuelis,* in addition to those animals found on the southern side. This difference can be explained by the fact that the sewer outfall opens offshore in a line with the rocky projection which separates the two sides. The current flows primarily from north to south carrying the polluted water in the direction of the southern section.

The point designated as L.A. 7 is located in the west channel of Los Angeles Harbor. The term "L.A. 7" was given to this particular point in a pollution survey conducted in 1952 by the Los Angeles Regional Water Pollution Control Board, and has been retained in this study so that this site may be recognized and related to data taken from that point. Specimens of *Pachygrapsus crassipes* tested were taken from the piling and floats at one of the small yacht harbors in this area. The nature of the pollution at this point has been designated as primarily raw sewage from approximately 500 persons. Oxygen content of the water, measured at monthly intervals over a period of 3 years (1956 through 1958), showed fluctuations from a low of 2.0 to a high of 8.2 parts per million. An ecological survey of the area shows the customary wharf fauna of the southern California waters. Anemones, the wharf mussel (*Mytilus edulis*), tunicates both solitary and colonial, hydroids, barnacles, and colonial serpulid worms are attached in great abundance to the underside of the wooden floats, while numerous speci-

mens of *Pachygrapsus crassipes* live on the floats and piling just above the water line. Since the area is maintained as a small yacht harbor, the floats are scraped occasionally and invariably are soon resettled by young forms of the species mentioned.

The conditions at the mouth of the San Gabriel River at Alamitos Bay were thoroughly investigated and reported by Reish in 1956. He reports the primary source of waste discharge into that region to be the Dow Chemical Company, the Santa Fe Springs Waste Disposal Company, two domestic sewage disposal plants, and the Los Angeles Bureau of Water and Power Steam Plant.

Since specimens of the same species living in the same conditions are known to have a consistent pattern of free amino acids in their muscle tissues, an investigation of these amino acids should indicate whether or not a change from a normal to an abnormal metabolism has taken place. An analysis of free amino acids was thus undertaken by means of two-dimensional paper chromatography.

Muscle tissue only was used for the determinations. All samples studied were taken from individual animals. Pooled samples were not used. Specimens were quick-frozen; muscle tissue from the foot of the abalone and from the legs of the crab was excised and subsequently lyophilized without thawing. This precaution was taken to inhibit the activity of autoenzymes and bacterial enzymes. After lyophilization the tissue was extracted with cold 70 per cent ethanol. Two 50 ml. aliquot portions were used, and extraction was permitted for at least 12

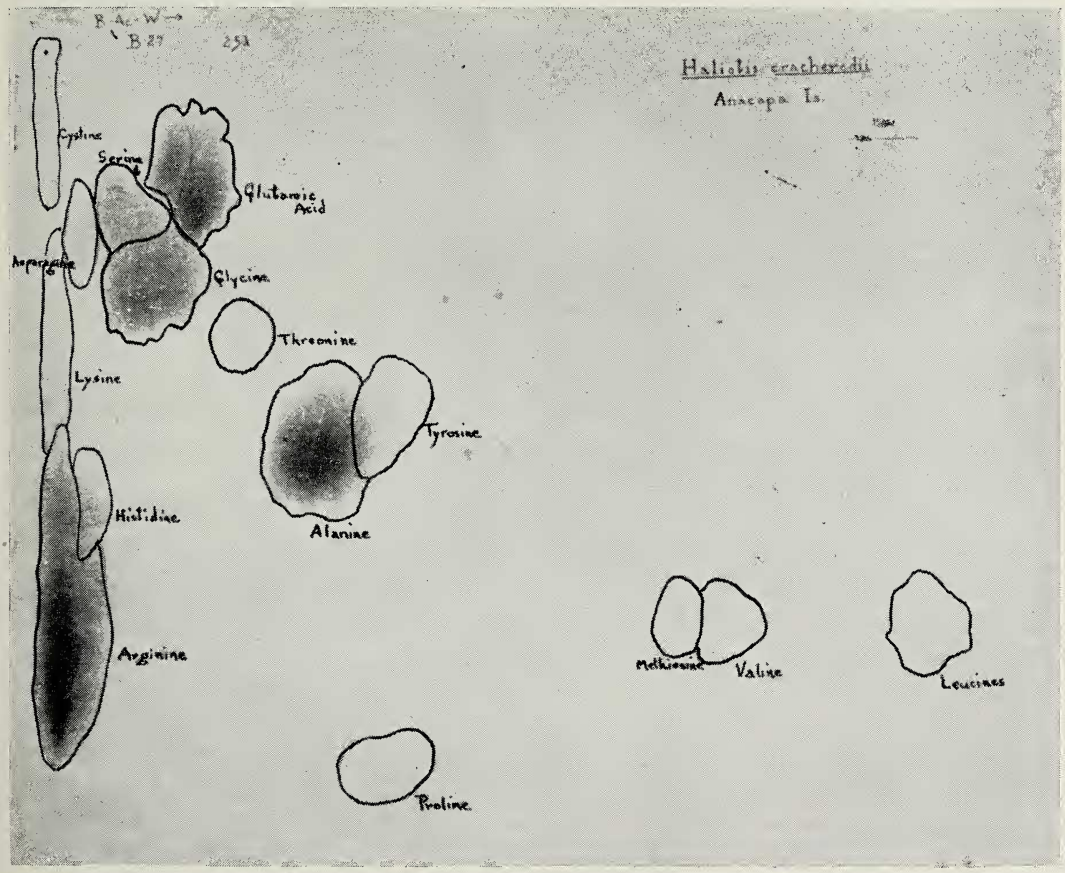

FIG. 2. Chromatogram of free amino acids of *Haliotis cracherodii* taken from Anacapa Island, a nonpolluted area.

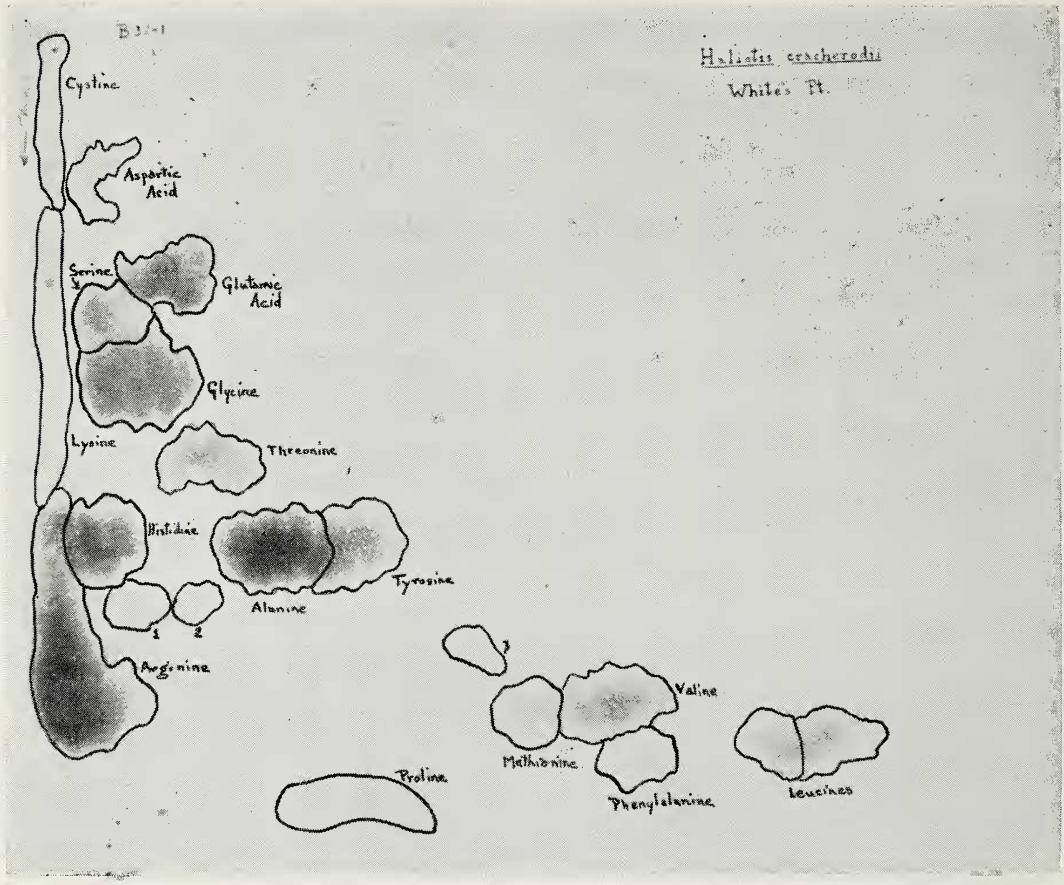

FIG. 3. Chromatogram of free amino acids of *Haliotis cracherodii* taken from White's Point, a polluted area.

hours for each portion. Extracts were then concentrated and stored under refrigeration as a 4.4 ml. solution of 10 per cent isopropanol.

Chromatograms were run on 18½- by 22¼-inch Whatman no. 1 filter papers. A mixture of butanol, acetic acid, and water (4:1:5) was used as the first phase; water-saturated phenol was used as the second. Development was carried out by dipping in a 0.2 per cent solution of ninhydrin in acetone.

RESULTS

The abalone, *Haliotis cracherodii,* from both polluted and nonpolluted areas contained the amino acids alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, leucine-isoleucine, tyrosine, and valine. Specimens taken from the nonpolluted areas (Santa Catalina Island, San Clemente Island, and Anacapa Island) contained asparagine in addition to the above amino acids. The specimens taken from White's Point showed no asparagine, but did show a definite spot identified as aspartic acid. This acid was either absent or only very faintly discernible in the specimens from the nonpolluted areas. Phenylalanine and three unidentified spots were present in the specimens obtained from the White's Point area (Figs. 2, 3; Table 1).

All specimens of the crab, *Pachygrapsus crassipes,* contained the free amino acids alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, leucine-isoleucine, lysine, methionine, proline, serine, threonine, tyrosine, and

valine. Specimens taken from Santa Catalina Island, Anacapa Island, and Morro Bay contained asparagine as well. Those from Santa Catalina and Anacapa islands contained an unidentified amino acid located to the right of arginine on the chromatographic pattern (Figs. 4, 5; Table 2).

DISCUSSION

Both *Haliotis cracherodii,* which occurs in one markedly polluted area, and *Pachygrapsus crassipes,* which was collected from a variety of polluted areas, have a free amino acid composition different from that of the same animals collected from nonpolluted areas. *Haliotis* from clean water gives a more consistent pattern than does *Pachygrapsus.* Both forms taken from polluted waters are marked by the absence of asparagine. This indicates that in some manner the metabolism dealing with this amino acid has been altered. In the case of *Haliotis* from White's Point, phenylalanine and three unidentified spots appeared, indicating a more extensive change in metabolic pattern. The change in the abalone is greater than that in the crab. This may be due either to a difference in response to polluted conditions because of the difference in the animals; or it may in some way be associated with the difference in length of time during which the animals are submerged and are subjected to the polluted conditions.

The factors of pollution common to the areas considered were a depletion of available oxygen and an increase in nutrients resulting

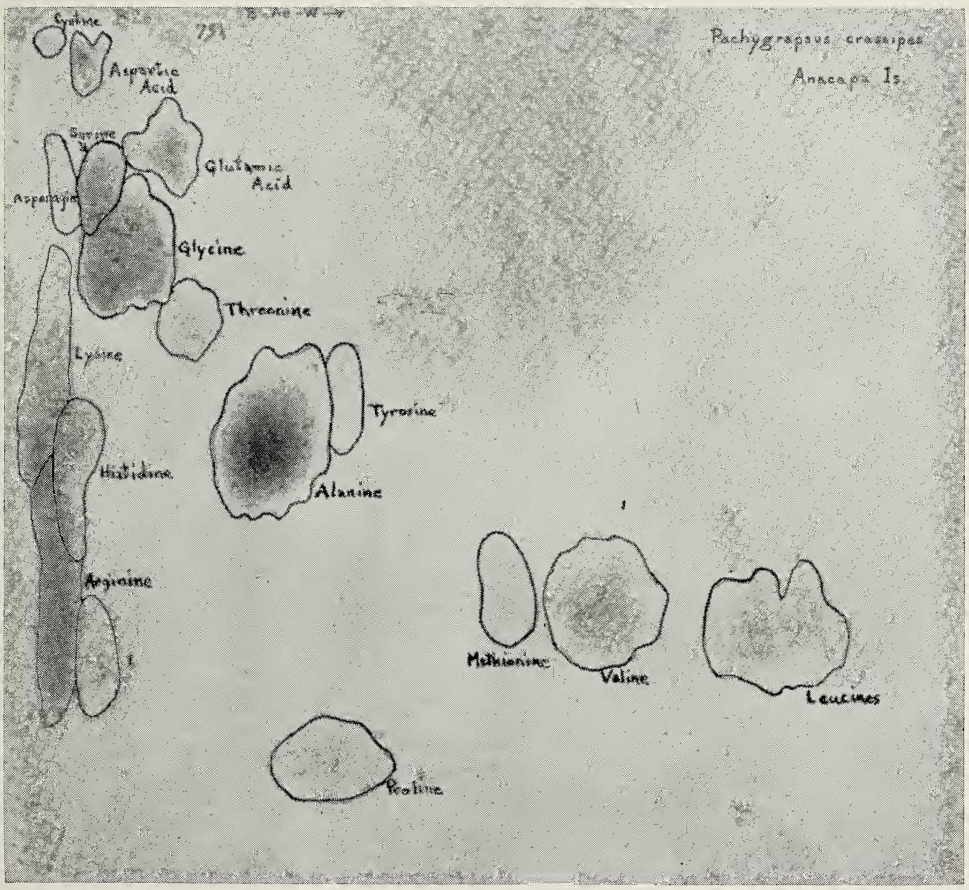

FIG. 4. Chromatogram of free amino acids of *Pachygrapsus crassipes* from Anacapa Island, a nonpolluted area.

TABLE 1

FREE AMINO ACIDS PRESENT IN SPECIMENS OF THE ABALONE, *Ha'iotis cracherodii*, FROM A POLLUTED AND THREE NONPOLLUTED AREAS

	ALANINE	ARGININE	ASPARTIC ACID	ASPARAGINE	CYSTINE	GLUTAMIC ACID	GLYCINE	HISTIDINE	LEUCINES	LYSINE	METHIONINE	PHENYLALANINE	PROLINE	SERINE	THREONINE	TYROSINE	VALINE	UNKNOWN #1	UNKNOWN #2	UNKNOWN #3
Santa Catalina Is.	×	×	×*	×	×	×	×	×	×	×	×		×	×	×	×	×			
Anacapa Is.	×	×		×	×	×	×	×	×	×	×		×	×	×	×	×			
San Clemente Is.	×	×		×	×	×	×	×	×	×	×		×	×	×	×	×			
White's Point	×	×	×		×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×

* Very faint.

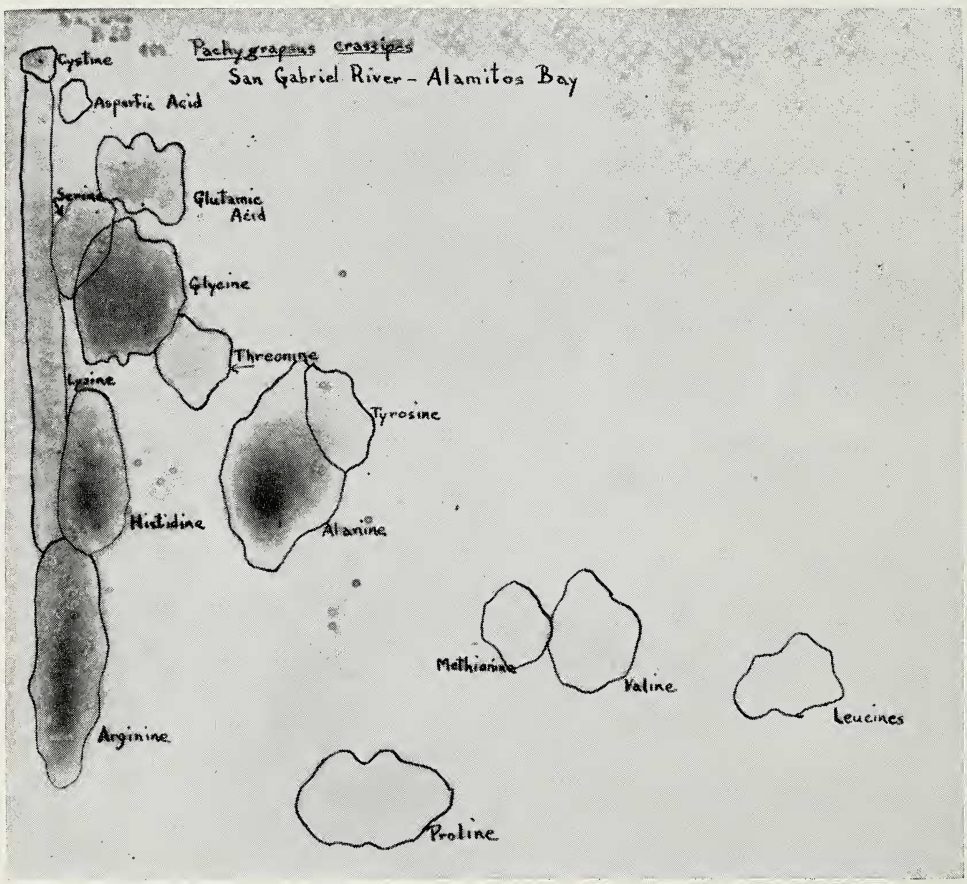

FIG. 5. Chromatogram of free amino acids of *Pachygrapsus crassipes* from the mouth of the San Gabriel River at Alamitos Bay, a polluted area.

TABLE 2

FREE AMINO ACIDS PRESENT IN SPECIMENS OF THE CRAB, *Pachygrapsus crassipes*, FROM FOUR NONPOLLUTED AND THREE POLLUTED AREAS

	ALANINE	ARGININE	ASPARTIC ACID	ASPARAGINE	CYSTINE	GLUTAMIC ACID	GLYCINE	HISTIDINE	LEUCINES	LYSINE	METHIONINE	PHENYLALANINE	PROLINE	SERINE	THREONINE	TYROSINE	VALINE	UNKNOWN #1
Santa Catalina Is.	×	×	×	×	×	×	×	×	×	×	×		×	×	×	×	×	×
Anacapa Is.	×	×	×	×	×	×	×	×	×	×	×		×	×	×	×		×
San Clemente Is.	×	×	×		×	×	×	×	×	×	×		×	×	×	×		
Morro Bay	×	×	×	×	×	×	×	×	×	×	×		×	×	×	×		×
White's Point	×	×	×		×	×	×	×	×	×	×		×	×	×	×		
L. A. Harbor	×	×	×		×	×	×	×	×	×	×		×	×	×	×		
Alamitos Bay	×	×	×		×	×	×	×	×	×	×		×	×	×	×		

from sewage. Since the role of the free amino acids in the animal body is as yet not completely understood, it is not possible to state what physiological mechanisms have been altered by these environmental changes or whether one or both of the factors have operated to bring about the altered free amino acid metabolism. That a biochemical change as well as a morphological change has occurred is evident.

SUMMARY

1. Specimens of the abalone, *Haliotis cracherodii,* and the crab, *Pachygrapsus crassipes,* were collected from clean and polluted waters.

2. Chromatographic analysis of the free amino acids of these forms were made by two-dimensional paper chromatography.

3. Specimens from polluted areas were found to be lacking in asparagine. This amino acid was present in specimens from clean waters.

4. Phenylalanine and three unidentified amino acids, not found in *Haliotis cracherodii* from clean water, were present in this species taken from polluted water.

5. Polluted waters differed from nonpolluted waters in that they showed a depletion of available oxygen and an increase in nutrients.

REFERENCES

KINNE, OTTO. 1958. Über die Reaktion erbgleichen Coelenteraten-gewebes auf verschiedene Salzgehalts und Temperaturbedigen, II. Mitteilung über den Einfluss des Salzgehaltes auf Wachstum und Entwicklung mariner, brackischer und limnischer Organismen. Zool. Jahrb. Abt. Zool. Physiol. Tiere 67(4): 407–488.

LANE, C. E., and R. D. SCHAFER. 1959. Work in progress.

REISH, D. J. 1956. An ecological study of lower San Gabriel River, California, with special reference to pollution. Calif. Fish and Game 42(1): 51–61.

——— 1959. Unpublished data. Personal communication.

STEPHENSON, R. E., and JOHN R. GRADY. 1956. Plankton and associated nutrients in the water surrounding three sewer outfalls in southern California. Report to Hyperion Engineers, Inc. Univ. of Southern Calif. Press. 48 pp.

WILSON, D. P., and F. A. J. ARMSTRONG. 1958. Biological differences between sea waters: Experiments in 1954 and 1955. Jour. Marine Biol. Assoc. United Kingdom 37(2): 331–348.